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(54) **Use of a microorganism or cell to induce autoimmunization of an organism against a tumor**

(57) The present invention relates to the use of a microorganism or cell containing a DNA sequence encoding a desired polypeptide or RNA to induce autoimmunization of an organism against a tumor. Furthermore, the present invention relates to a method for the production of antibodies against a tumor comprising (a) injecting a microorganism or cell containing a DNA sequence encoding a desired polypeptide or RNA into an organism bearing a tumor and (b) isolating antibodies against the tumor.

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Description

[0001] The present invention relates to the use of a microorganism or cell containing a DNA sequence encoding a desired polypeptide or RNA to induce autoimmunization of an organism against a tumor. Furthermore, the present invention relates to a method for the production of antibodies against a tumor comprising (a) injecting a microorganism or cell containing a DNA sequence encoding a desired polypeptide or RNA into an organism bearing a tumor and (b) isolating antibodies against the tumor.

[0002] The present applicant found that the method described in EP 03 018 478.2 relating to "The production of a polypeptide, RNA or other compound in a tumor tissue" also enables the production of antibodies against the tumor tissue. These antibodies present an autoimmunization of the organism bearing the tumor. Furthermore, these antibodies can be isolated and used for the treatment of tumors in other organisms. The term "organisms" refers to animals and human beings. The disclosure content of EP 03 018 478.2 is also part of the present application.

[0003] In the following the present application is exemplarily described in the reduction and elimination of xenogeneic GI-101A solid breast carcinoma tumors and their metastases in nu/nu mice (T cell deficient mice).

Step#1: Female nu/nu mice of 5 weeks age were chosen, and the GI-101A cells grown in RPMI1640 medium, supplemented with estrogen and progesterone. When reached desired confluence, cells were harvested, washed with phosphate buffered saline. Cells (5×10^6 cells per mouse) were then injected subcutaneously into mice. The tumor growth was carefully monitored every two days.

Step#2: At two stages of tumor growth (at tumor size of 400-600 mm^3, and at tumor size of ~ 1700 mm^3), purified vaccinia viral particles were delivered to each tumorous mice by intravenous injection through tail vein. The colony purified virus was amplified in CV-1 cell line and the intracellular viral particles were purified by centrifugation in sucrose gradient. Two concentrations of virus (10^6 pfu/100 □ and 10^7 pfu/100 □ resuspended in PBS solution) were injected. The viral replication was monitored externally by visualization of virus-mediated green fluorescence protein expression. The tumor development was monitored by tumor volume determination with a digital caliper.

Step#3: After viral application, it was determined that first the tumors continued to grow to a size of ~ 900 mm^3 (from 400-600 mm^3 at the time of viral injection), and to a size of ~ 2400 mm^3 (from 1700 mm^3). Then the growth rate leveled off for approximately 6-8 days.

Step#4: Approximately 14 days after viral injection, the tumor volume started to decline rapidly (Figure 1). Forty days after viral application, all the treated animals showed more than 60% tumor regression. Sixty-five days after viral treatment and many of the animals had complete regression of tumors.

Step#5: Some of the animals were completely tumor-free for several weeks and their body weight returned to normal.

Step#6: The level of immune activation was determined. According to the immunoblot analysis of the table 1 sera were obtained from the animals with regressing tumors and the immune titer determined against a foreign protein (e.g. green fluorescent protein), vaccinia viral proteins, and GI-101A cancer cell proteins.

[0004] In conclusion, the present invention shows that solid tumors allowed an enormous tumor-specific vaccinia virus replication, which led to tumor protein antigen and viral protein productions in the tumors. In addition, vaccinia virus did lyse the infected tumor cells and thereby released tumor-cell-specific antigens. The continuous leakage of these antigens into the body led to a very high level of antibody titer (in approximately 7-14 days) against foreign cell proteins (tumor proteins), viral proteins, and the virus encoded engineered proteins in the mouse body. The newly synthesized antitumor antibodies and the enhanced macrophages, neutrophils counts were continuously delivered via the vasculature into the tumor and thereby provided for the recruitment of an activated immune system in the inside of the tumor. The active immune system then eliminated the foreign compounds of the tumor including the viral particles. This interconnected release of foreign antigens boosted in antibody production and continuous return of the antibodies against the tumor-contained proteins function like an autoimmunization vaccination system initiated by vaccinia viral replication, followed by cell lyses, protein leakage and enhanced antibody production (see figure 2).

[0005] Thus, the present invention teaches a complete process which may be applied to all tumor systems with immunoprivileged tumor sites as site of privileged viral, bacterial, and mammalian cell growth. These findings may lead the way for tumor elimination by the host own immune system.

TABLE 1:

[0006] Immunoblot analysis. The antisera obtained from the following sources are used to analyze the following listed samples.

Samples:

1). Mouse cell lysate (control);
2). Purified and denatured vaccinia viral particles;
3). GI-101A tumor cell lysate;
4). Purified green fluorescent protein;
5). Purified luciferase protein;
6). Purified beta-galactosidase protein.

Antisera:

a). Antiserum from nontumorous mouse;

b). Antiserum from GI-101A tumorous mouse;
c). Antiserum from GI-101A tumorous mouse 14 days after vaccinia i.v. injection;
d). Antiserum from GI-101A tumorous mouse 65 days after vaccinia i.v. injection;
e). Antiserum from tumor-free mouse (after elimination of GI-101A tumor) 80 days after vaccinia i.v. injection.

BRIEF DESCRIPTION OF THE DRAWINGS:

[0007]

Figure 1. Tumor size reduction. Vaccinia viruses RVGL-12+GCV(gancyclovir), and RVGL-12 were injected on 67 days after GI-101A cellular implantation.. RVGL-12a virus was injected on 30 days after cellular implantation. RVGL-12+GCV treatment resulted in 86.3% reduction of tumor size (Day 52 after viral injection) from their peak volumes on Day 13. RVGL-12 treatment resulted in 84.5% reduction of tumor size (Day 52) from their peak volumes (Day 13). RVGL-12a treatment resulted in 98.3% reduction of tumor size (Day 89) from their peak volumes (Day 12). On the contrary, with PBS+GCV control treatment, the average volume of tumors were increased by 91.8% in 38 days.

Claims

1. Use of a microorganism or cell containing a DNA sequence encoding a desired polypeptide or RNA to induce autoimmunization of an organism against a tumor.

2. Method for the production of antibodies against a tumor comprising (a) injecting a microorganism or cell containing a DNA sequence encoding a desired polypeptide or RNA into an organism bearing a tumor and (b) isolating antibodies against the tumor.

FIGURE 1

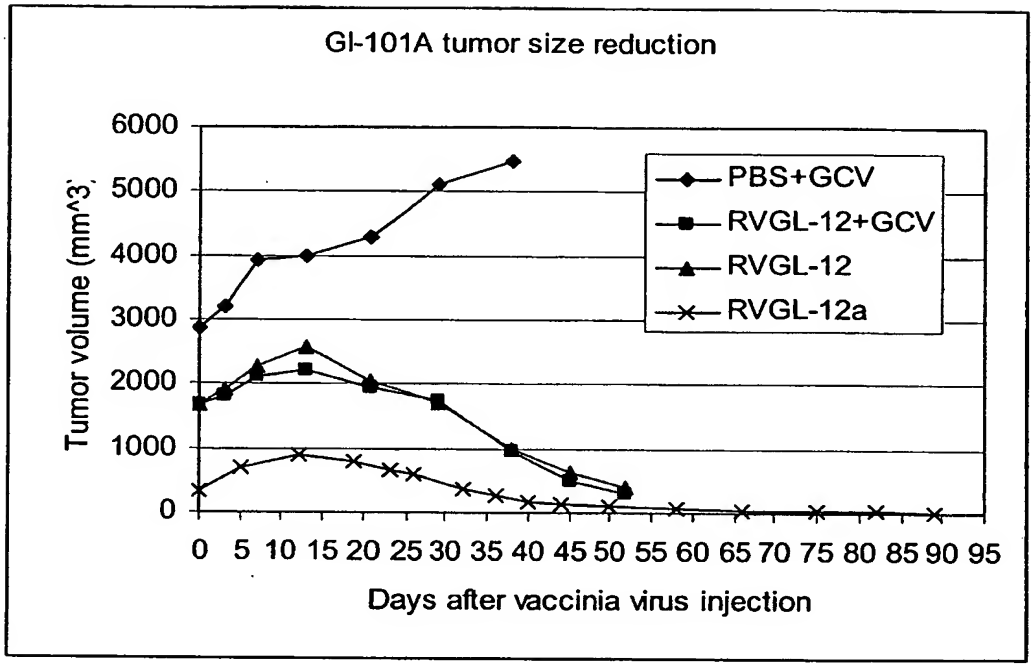
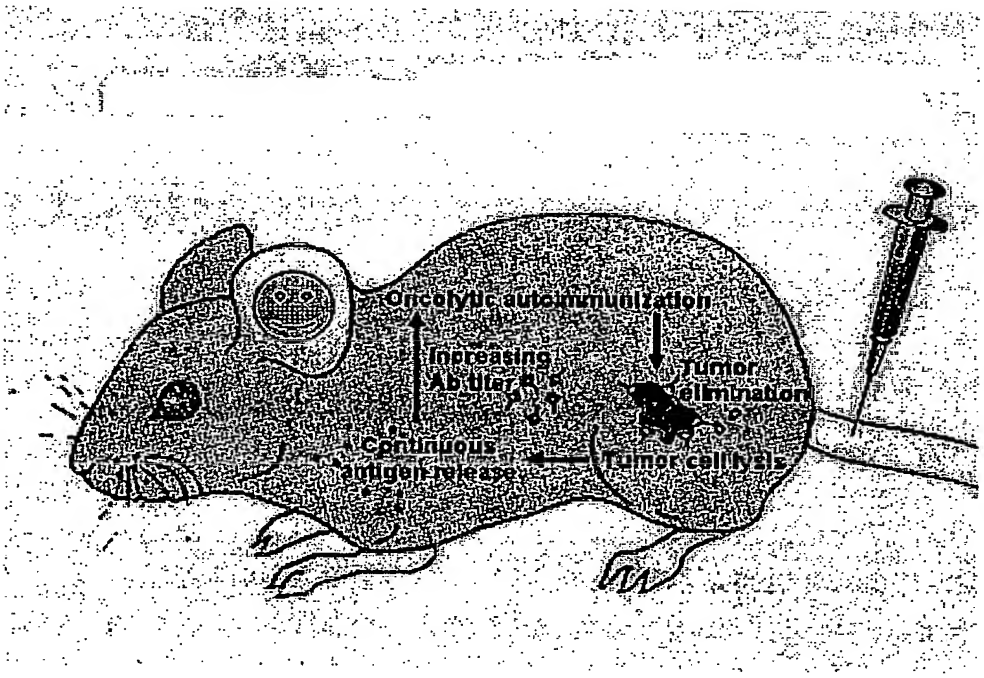

GI-101A tumor size reduction
Tumor volume (mm^3)
6000
5000
4000
3000
2000
1000
0
0 5 10 15 20 25 30 35 40 45 50 55 60 65 70 75 80 85 90 95
Days after vaccinia virus injection
PBS+GCV
RVGL-12+GCV
RVGL-12
RVGL-12a

Figure 2. Autoimmunization scheme.



European Patent Office

PARTIAL EUROPEAN SEARCH REPORT

which under Rule 45 of the European Patent Convention shall be considered, for the purposes of subsequent proceedings, as the European search report

Application Number EP 03 02 4283

DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int.Cl.7)
X	KAUFMAN H ET AL: "A RECOMBINANT VACCINIA VIRUS EXPRESSING HUMAN CARCINOEMBRYONIC ANTIGEN CEA" INTERNATIONAL JOURNAL OF CANCER, vol. 48, no. 6, 1991, pages 900-907, XP009023961 ISSN: 0020-7136 * page 904, column 2, paragraph 2 - paragraph 5; figures 7,8; table 1 * ---	1,2	C12N15/86 A61K48/00 A61K39/00
X	WO 96 11279 A (US HEALTH) 18 April 1996 (1996-04-18) * page 32, line 5 - page 33, line 10; claims 16,17; example 4 * --- -/--	1,2	
			TECHNICAL FIELDS SEARCHED (Int.Cl.7)
			C12N A61K

INCOMPLETE SEARCH

The Search Division considers that the present application, or one or more of its claims, does/do not comply with the EPC to such an extent that a meaningful search into the state of the art cannot be carried out, or can only be carried out partially, for these claims.

Claims searched completely :

Claims searched incompletely :

Claims not searched :

Reason for the limitation of the search:

see sheet C

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Place of search	Date of completion of the search	Examiner
MUNICH	21 January 2004	Vandenbogaerde, A

CATEGORY OF CITED DOCUMENTS

X : particularly relevant if taken alone
Y : particularly relevant if combined with another document of the same category
A : technological background
O : non-written disclosure
P : intermediate document

T : theory or principle underlying the invention
E : earlier patent document, but published on, or after the filing date
D : document cited in the application
L : document cited for other reasons

& : member of the same patent family, corresponding document

EPO FORM 1503 03.82 (P04C07)

European Patent Office

PARTIAL EUROPEAN SEARCH REPORT

Application Number
EP 03 02 4283

DOCUMENTS CONSIDERED TO BE RELEVANT			CLASSIFICATION OF THE APPLICATION (Int.Cl.7)
Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	
X	OVERWIJK W W ET AL: "Vaccination with a recombinant vaccinia virus encoding a self antigen induces autoimmune vitiligo and tumor cell destruction in mice: requirement for CD4(+) T lymphocytes" PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF USA, NATIONAL ACADEMY OF SCIENCE. WASHINGTON, US, vol. 96, no. 6, March 1999 (1999-03), pages 2982-2987, XP002226929 ISSN: 0027-8424 * abstract * ---	1,2	
X	US 6 589 531 B1 (MCALLISTER-MORENO ANDRES ET AL) 8 July 2003 (2003-07-08) * column 18, line 30 - line 55 * * column 12, line 62 - column 13, line 39 * -----	1,2	TECHNICAL FIELDS SEARCHED (Int.Cl.7)

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EPO FORM 1503 03.82 (P04C10)

European Patent Office

INCOMPLETE SEARCH SHEET C

Application Number
EP 03 02 4283

Although claims 1-2 are directed to a method of treatment of the human/animal body (Article 52(4) EPC), the search has been carried out and based on the alleged effects of the compound/composition.

Reason for the limitation of the search:

Present claims 1-2 relate to the use of an extremely large number of possible microorganisms or cells containing an extremely large number of possible DNA sequences encoding a desired polypetide or RNA for the treatment of cancer or for the production of antibodies. Support within the meaning of Article 84 EPC and/or disclosure within the meaning of Article 83 EPC is to be found, however, for only a very small proportion of the microorganisms or cells claimed. In the present case, the claims so lack support, and the application so lacks disclosure, that a meaningful search over the whole of the claimed scope is impossible. Consequently, the search has been carried out for those parts of the claims which appear to be supported and disclosed, namely those parts relating to use of vaccinia virus as cancer vaccine or for the production of antibodies.
Moreover, the meaning of the recombinant vaccinia virus (RVGL) used in the example is not clear.

ANNEX TO THE EUROPEAN SEARCH REPORT ON EUROPEAN PATENT APPLICATION NO. EP 03 02 4283

This annex lists the patent family members relating to the patent documents cited in the above-mentioned European search report. The members are as contained in the European Patent Office EDP file on
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21-01-2004

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		JP 2003520597 T	08-07-2003
		WO 0153467 A1	26-07-2001
		US 2003157128 A1	21-08-2003

EPO FORM P0459

For more details about this annex : see Official Journal of the European Patent Office, No. 12/82